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DEVELOPMENT AND VALIDATION OF SPECTROSCOPIC METHODS FOR THE SIMULTANEOUS ESTIMATION OF RASAGILINE AND RILUZOLE

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Keywords:

Rasagiline, Riluzole, Spectroscopic method, Absorption, Simultaneous estimation, Methanol

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ABSTRACT: In the present study, an effort has been made to estimate Rasagiline and Riluzole simultaneously in their bulk and physical mixture by UV-Visible spectrophotometric methods. The methods developed are precise, reliable simultaneous equations and area under the curve (AUC) method. The absorption maxima in the simultaneous equation method are 265.20 nm and 263.40 nm, respectively, for rasagiline and riluzole. Similarly, in the area under the curve method, the wavelength selected for estimation is in the range of 260.20 - 270.20 nm and 258.40-268.40 nm, respectively for rasagiline and riluzole. In the two methods developed, the Beer- Lambartz's range was in the concentration range of 50 to 250 µg/mL for rasagiline and 4 to 20µg/mL for riluzole. The percentage recovery was found to be in the range of 100.43% and 100.01% for rasagiline and riluzole. ICH guidelines were followed in the proposed methods for their reproducibility and accuracy. The proposed methods can be adopted industrially and in research laboratories for the routine simultaneous analysis of Rasagaline and Riluzole in their bulk and dosage forms.

INTRODUCTION: Rasagiline is chemically (1R)-N-(prop-2-yn-1-yl)-2, 3-dihydro-1H-inden-1-amine (Fig:1) Its molecular formula is $C_{12}H_{13}N$ and molecular weight is 171.23 g/mol. It is soluble in Methanol. It is used in the treatment of Amyotrophic lateral sclerosis (ALS) and belongs to the category of an antiparkinsonian agent [1, 2]. Rasagiline is a propargyl amine and an irreversible inhibitor of monoamine oxidase (MAO). MAO, a flavin-containing enzyme, regulates the metabolic degradation of catecholamines and serotonin in the CNS and peripheral tissues. MAO-B is the major form in the human brain and is responsible for the regulation of the metabolic degradation of dopamine and phenylethylamine.

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Rasagiline was also shown to be a potent and irreversible inhibitor of MAO-B in platelets [3, 4, 5].

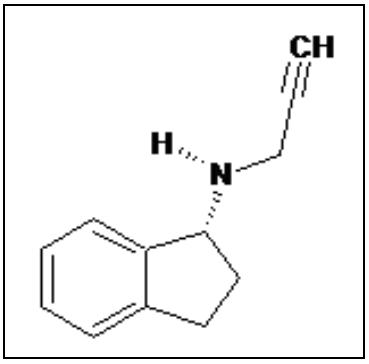

FIG. 1: STRUCTURE OF RASAGILINE

Riluzole is chemically 6-(trifluoromethoxy)-1,3-benzothiazol-2-amine. Its molecular formula is $C_8H_5F_3N_2OS$, and its molecular weight is 234.198 g/mol[6]. It is slightly soluble in water but completely soluble in methanol [7, 8, 9, 10, 11]. It is used in the treatment of amyotrophic lateral sclerosis (ALS) and belongs to the category of anti-convulsant.

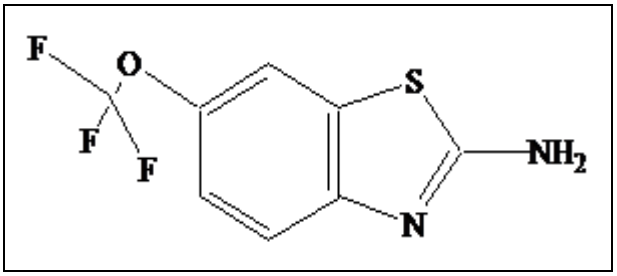

FIG. 2: STRUCTURE OF RILUZOLE

It is found that the combination of rasagiline with riluzole is safe and increases survival by about 20% in a dose-dependent manner for the improvement of current neuroprotective treatment strategies of ALS [2, 12]. Validated analytical methods are required to characterize these drugs and product composition in all the phases of their formulation developments. On the literature survey, it is revealed that several methods of analyzing rasagiline [13, 14, 15,] and riluzole [16, 17] by UV-Visible spectrophotometric methods were found. But no analytical method was found for simultaneous estimation of rasagiline and riluzole. Keeping these observations in mind, we have decided to develop newer, simpler, rapid, accurate, and reproducible spectrophotometric methods for simultaneous estimation of rasagiline and riluzole in bulk and pharmaceutical dosage form. A physical mixture was prepared by blending each 50 mg of rasagiline and riluzole. Tablets were prepared from the mixture with other excipients required for tablet formulation as no combined marketed dosage forms are found.

MATERIALS AND METHODS:

Materials: All the chemicals and reagents used for the development of proposed methods to estimate rasagiline and riluzole are of spectroscopic grade. The instrument UV-Visible spectrophotometer (Shimadzu-1800) was used for the analytical method development and validation of rasagiline and riluzole. The present work was carried out at the Department of pharmaceutical chemistry, Govt. College of Pharmacy, Bengaluru. A pure sample of rasagiline and riluzole for the current study was procured from reliable sources. Rasagiline was purchased from Subtle Pharmaceuticals Pvt Ltd., and riluzole was purchased from Trichemie pharma., methanol (HPLC grade) from Merck. Double distilled Millipore water was used for the analysis, and the same was collected from Milli pore Direct Q3.

Methods: The following spectrophotometric methods were selected to analyze the combination of rasagiline and riluzole, namely,

Method A: Simultaneous equation method
Method B: Area under the curve (AUC) method

The instrumental specifications of the UV-Visible spectrophotometer used to perform the above methods are in **Table 1**.

TABLE 1: INSTRUMENTAL SPECIFICATIONS

UV-Visible spectrophotometer	SHIMADZU 1800
Software	UV Probe Version 2.43
Balance	Sartorius

Method A: Simultaneous Equation Method:

Selection of Solvent for Analysis: The selection of solvents for analysis was carried out by the effect of different solvents on the pure drug and tablet powder. Riluzole was slightly soluble in water. But both the drugs are soluble in methanol and were stable. Hence, methanol was chosen for the preparation of solutions for analysis.

Selection of Analytical Wavelengths: Standard stock solutions having a concentration of 10 µg/mL was prepared separately, and they were scanned in the wavelength range of 200-400 nm. The maximum (λ_{max}) absorbance of both the drugs was found to be 265.20 nm for rasagiline and 263.40 nm for riluzole.

Preparation of Standard Stock Solutions and Calibration Curve: The stock solution was prepared by dissolving each 50 mg of accurately weighed rasagiline and riluzole in two different 50 mL standard volumetric flask and the final volume was adjusted to 50 mL with methanol to give the stock solution of 1000 µg/mL (stock A) concentration. From stock A, solution of riluzole 2.5 mL was placed in 25 mL volumetric flask, and volume was adjusted with methanol to give a solution of 100 µg/mL of riluzole (stock B). From rasagiline stock solution A 0.5-2.5 mL and 0.4-2 mL of riluzole stock B were pipetted in to 10 mL of volumetric flasks, volume was made up with methanol to get concentrations of 50-250 µg/mL of rasagiline and 4-20 µg/mL of riluzole respectively. The absorbance of the resulting solution was measured against the wavelength of 265.20 nm and 263.40 nm, respectively, against the blank methanol **Fig. 3**, **4**.

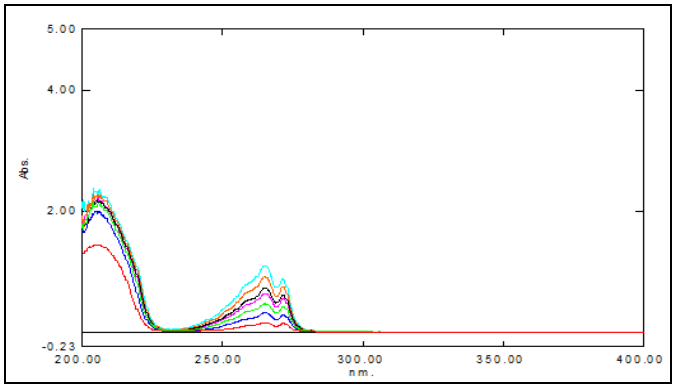

FIG. 3: UV-VISIBLE SPECTRA OF RASAGILINE AT 50-250 µg/mL

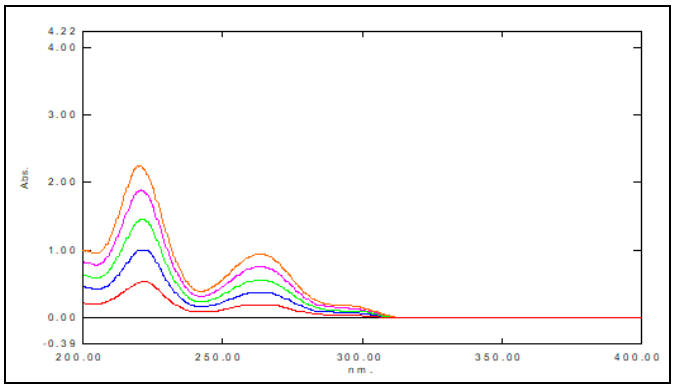

FIG. 4: UV- VISIBLE SPECTRA OF RILUZOLE AT 4-20 µg/mL

Determination: Simultaneous equation method can be used to determine the concentration of two drugs in combination if each of the drugs absorbs at the λ_{max} of the other. The two wavelengths selected for the development of simultaneous equations are 265.20 nm and 263.40 nm. The formula for simultaneous estimation of drugs used is

$$C_X = A_2a_{y1}-A_1a_{y2} / a_{x2}a_{y1}-a_{x1}a_{y2} \quad \text{Or}$$

$$C_X = A_1a_{y2}-A_2a_{y1} / a_{x1}a_{y2} - a_{x2}a_{y1}$$

$$C_y = A_1a_{x2}-A_2a_{x1} / a_{x2}a_{y1}-a_{x1}a_{y2} \quad \text{Or}$$

$$C_y = A_2a_{x1}-A_1a_{x2} / a_{y2}a_{x1}-a_{y1}a_{x2}$$

Where,

C_X = concentration of rasagiline in mixture

C_Y = concentration of riluzole in the mixture

ax_1 and ax_2 are absorptivity of rasagiline at its λ_{max} and riluzole λ_{max}

ay_1 and ay_2 are absorptivity of riluzole at its λ_{max} and rasagiline λ_{max}

The absorptivity values are determined by $E^{1\%}_{1cm}$. The absorbance values of rasagiline at 265.20 nm and 263.40 nm, riluzole at 265.20 nm, and 263.40 nm are in **Table 2** & **3,** and absorbance of their mixture is in **Table 4**.

TABLE 2: ABSORBANCE OF RASAGILINE AT 265.20 nm AND 263.40 nm

S. no.	Concentration of rasagiline (µg/mL)	Absorbance		$E^{1\%}1cm$	
		265.20 nm	263.40 nm	265.20 nm	263.40 nm
1	50	0.15	0.14	30	28
2	100	0.32	0.29	32	29
3	150	0.47	0.43	31.33	28.66
4	200	0.62	0.58	31	29
5	250	0.77	0.66	30.8	26.4
			Average	31.026	28.212

Here, ax1 =31.026, ax2 =28.212

TABLE 3: ABSORBANCE OF RILUZOLE AT 265.20 nm AND 263.40 nm

S. no.	Concentration of rasagiline (µg/mL)	Absorbance		$E^{1\%}1cm$	
		265.20 nm	263.40 nm	265.20 nm	263.40 nm
1	4	0.191	0.19	477.5	475
2	8	0.371	0.37	463.75	462.5
3	12	0.552	0.56	460	466.66
4	15	0.75	0.75	468.75	468.75
5	20	0.933	0.94	466.5	470
			Average	467.3	468.582

Here a_{y1}=467.3 a_{y2}=468.582

TABLE 4: ABSORBANCE OF MIXTURE (RASAGILINE AND RILUZOLE)

S. no.	Concentration of rasagiline and riluzole (mix in µg/mL)		Absorbance		Concentration obtained (µg/mL)	
	Rasagiline	Riluzole	265.20 nm	263.40 nm	Rasagiline	Riluzole
1	50	4	0.1784	0.164	51.35	4.018
2	100	8	0.3568	0.328	102.716	8.156
3	150	12	0.5352	0.492	154.0744	12.231
4	200	16	0.7136	0.656	205.4321	16.313
5	250	20	0.892	0.82	256.7905	20.388

Calculation: From the above **Table 2** & **3**, a_{x1} = 31.026, a_{x2} = 28.212, a_{y1} = 467.3, a_{y2} = 468.58 A_1=Absorbance of mixture at λ_{max} 265.20 nm and A_2=Absorbance of mixture at λ_{max} 263.40 nm

In mixture 1:

A_1= 0.1784, A_2=0.164 (from **Table 4**)

a_{x1} = 31.026, a_{x2} = 28.212, a_{y1} = 467.3, a_{y2} = 468.58

$C_{Rasagiline}$ = (0.164 × 467.3 – 0.1784 × 468.58) / (28.212 × 467.3-31.026 × 468.58)
= 6.9574/1354.6954
= 0.005135mg/mL or 51.357 µg/mL

Similarly, $C_{Riluzole}$ = (0.1784×28.212-0.164 × 31.026) / 28.212 × 467.3-31.026 × 468.58)
= 0.0552
= 0.00047471mg/mL or 4.747µg/mL

For other mixture the calculations were done similarly as mentioned above

Method B: Area Under Curve Method (AUC):

Selection of Analytical Wavelengths: Standard stock solutions having the concentration of 10 µg/mL was prepared separately, and they were scanned in the wavelength range of 200-400 nm and the maximum (λ_{max}) absorbance of both the drugs were found to be 265.20 nm for rasagiline and 263.40 nm for riluzole.

Preparation of Standard Stock Solution: Stock solutions of rasagiline and riluzole were prepared as discussed in method A. From rasagiline stock solution 0.5-2.5 mL and 0.4-2 mL of riluzole stock B were pipetted into 10 mL of volumetric flasks, volume was made up with methanol to get concentrations of 50-250 µg/mL of rasagiline and 4-20 µg/mL of riluzole respectively. The absorbance of the resulting solution was measured against the wavelength of 265.20 nm and 263.40 nm, respectively, against the blank methanol. The area under the curve of rasagiline and riluzole are recorded in **Fig. 4** & **5**.

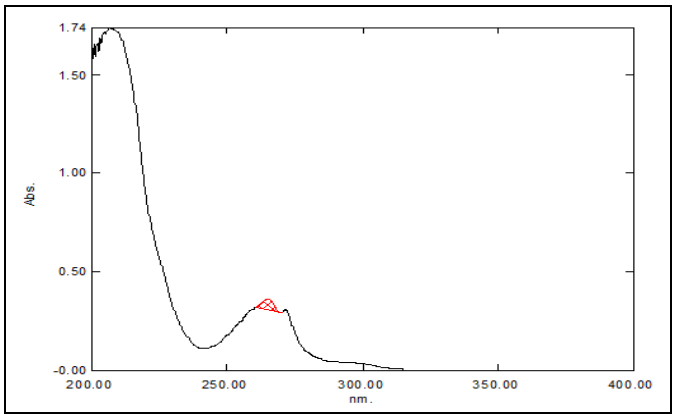

FIG. 4: AREA UNDER CURVE FOR RASAGILINE

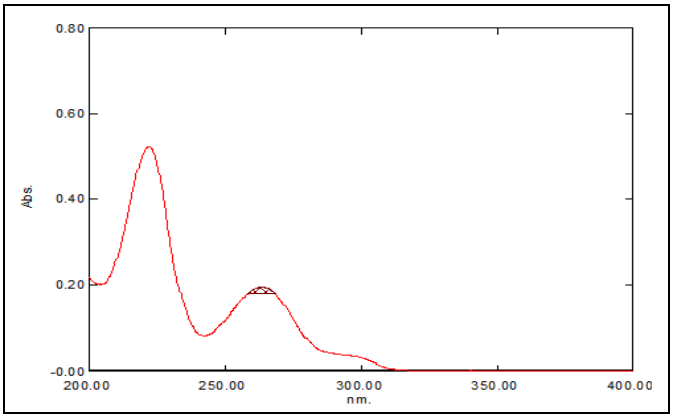

FIG. 5: AREA UNDER CURVE FOR RILUZOLE

Determination: Area under the curve method is mainly used for the quantification of the drugs in both single and multi-component formulation. It involves calculations of integrated values of absorbance with respect to wavelength in the indicated range.

The formula used to determine the concentration by area under the curve method is

$C_X = A_2a_{y1}-A_1a_{y2}/ a_{x2}a_{y1}-a_{x1}a_{y2}$ or

$C_X = A_1a_{y2}-A_2a_{y1}/a_{x1}a_{y2} -a_{x2}a_{y1}$

$C_y=A_1a_{x2}-A_2a_{x1}/a_{x2}a_{y1}-a_{x1}a_{y2}$ or

$C_y= A_2a_{x1}-A_1a_{x2}/a_{y2}a_{x1}-a_{y1}a_{x2}$

Where,
C_X= concentration of rasagiline in mixture
C_Y= concentration of riluzole in mixture
A_1 and A_2 are the areas under the curve of the sample mixture at 260.20 nm to 270.20 nm and 258.40 nm to 268.40 nm, respectively.
ax_1 and ax_2 are absorptivity of rasagiline and riluzole at 260.20 nm to 270.20 nm.
ay_1 and ay_2 are absorptivity of rasagiline and riluzole at 258.40 nm to 268.40 nm

The absorptivity values are determined by $E^{1\%}_{1cm}$.

Absorptivity ($E^{1\%}_{1cm}$) = (Area under curve /concentration in µg/ml) × 10000

The calibration data and calibration graph of rasagiline at 260.20 nm to 270.20 nm and 258.40 to 268.40 nm are in **Tables 5** and **6**, **Fig. 6** and **7**. Absorbance of the mixture (rasagiline and riluzole) is in **Table 7**.

TABLE 5: AREA UNDER CURVE OF RASAGILINE AT 260.20 -270.20 nm AND 258.40-268.40 nm

S. no.	Concentration of rasagiline (µg/mL)	Area under curve (AUC)		$E^{1\%}$1cm	
		260.20-270.20 nm	258.40-268.40 nm	260.20-270.20 nm	258.40-268.40 nm
1	50	0.15	0.21	30	42
2	100	0.31	0.43	31	43
3	150	0.46	0.65	30.66	43.33
4	200	0.64	0.88	32	44
5	250	0.74	1.00	29.6	40
		Average		30.652	42.466

Here ax_1=30.652, ax_2 =42.466

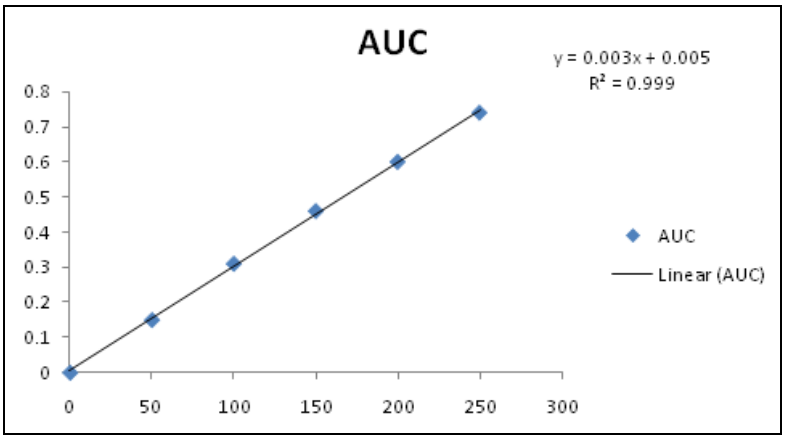

FIG. 7: CALIBRATION GRAPH OF RILUZOLE

TABLE 7: AREA UNDER CURVE DATA OF RASAGILINE AND RILUZOLE

S. no.	Concentration of rasagiline and riluzole (mixture µg/mL)		AUC absorbance		Concentration obtained (µg/mL)	
	Rasagiline	Riluzole	260.20-270.20 nm	258.40-268.40 nm	Rasagiline	Riluzole
1	50	4	0.26	0.32	51.55	4.0354
2	100	8	0.55	0.67	103.32	9.226
3	150	12	0.78	0.96	154.65	12.022
4	200	16	1.04	1.28	206.201	16.029
5	250	20	1.31	1.6	249.552	21.331

Calculation:

From the above **Table 5** & **6**, ax_1 = 30.652, ax2 = 42.466, ay_1 = 254.5, ay2 = 252.25,

A_1 = AUC of mixture at 260.20-270.20 nm, A_2 = AUC of mix at 258.40-268.40 nm

In mixture 1: A_1= 0.26, A_2 =0.32 (from **Table 7**)

$C_{Rasagiline}$ = (0.32 × 254.5 - 0.26 × 252.25) / (42.466 × 254.5 - 30.652 × 252.25)

= 15.855/3075.63

= 0.005155 mg/mL or 51.55 µg/mL

Similarly, $C_{Riluzole}$ = (0.26 × 42.466 - 0.32 × 30.652) / (42.466 × 254.5 - 30.652 × 252.25)

= 1.24116/3075.63

= 0.0004035mg/mL or 4.035µg/mL

For other mixtures, the calculations were done similarly as mentioned above.

Method validation: The developed method were validated according to their analytical procedures as per ICH guidelines for validation of analytical procedures in order to determine linearity, precision, LOD, LOQ, and accuracy for the analyte.

Linearity: The linearity of an analytical procedure is its ability (within a given range) to obtain test results that are directly proportional to the concentration of analyte in the sample solution.

Preparation of Working Standards of Rasagiline and Riluzole: Working standard stock solutions of rasagiline and riluzole were prepared as discussed in method A. From rasagiline stock solution 0.5-3.5 mL and 0.4-2 mL of riluzole stock B were pipetted into 10 mL of volumetric flasks, volume was made up with methanol to get concentrations of 50-350 µg/mL of rasagiline and 4-20 µg/mL of riluzole respectively.

The absorbance of the resulting solution was measured against the wavelength of 265.20 nm and 263.40 nm respectively against the blank methanol.

The linearity data and absorbance of rasgiline are in **Table 8** and **Fig. 8**. Similarly, for riluzole in **Table 9** and **Fig. 9**.

TABLE 8: LINEARITY DATA OF RASAGILINE IN METHANOL

S. no.	Concentration (µg/mL)	Absorbance	$E^{1\%}$ 1cm
1	50	0.15	30
2	100	0.28	28
3	150	0.41	27.33
4	200	0.54	27
5	250	0.67	26.8
6	300	0.8	26.6
7	350	0.91	26

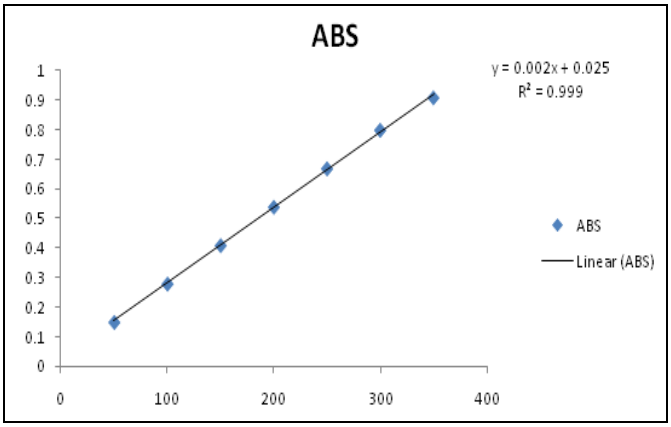

FIG. 8: ABSORBANCE LINEARITY OF RASAGILINE

TABLE 9: LINEARITY DATA OF RILUZOLE IN METHANOL

S. no.	Concentration (µg/mL)	Absorbance	$E^{1\%}$ 1cm
1	4	0.183	457.5
2	8	0.355	443.75
3	12	0.513	427.5
4	16	0.724	452.5
5	20	0.913	456.5

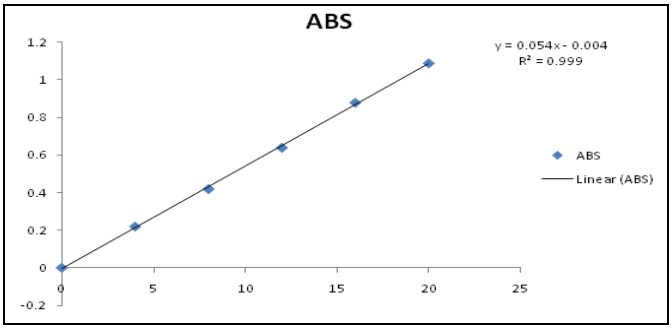

FIG. 9: ABSORBANCE LINEARITY OF RILUZOLE

Precision: Precision studies are carried out to ascertain the reproducibility of the proposed methods. Repeatability was determined by preparing six replicates of the same concentration of the sample, and the absorbance was measured.

A stock solution was prepared by dissolving 50 mg of accurately weighed rasagiline and riluzole into 50 mL volumetric flask, and the final volume was adjusted to 50 mL with methanol to get the stock solution 1000 µg/mL (stock A) concentration. From the resulting solution 2.5 mL of riluzole was placed in 25 mL volumetric flask and volume adjusted with methanol to give a solution of 100 µg/mL of riluzole solution (stock B). From stock solution, A 3.0 mL of rasagiline and 1.2 mL of riluzole of stock B were pipetted into 10 mL volumetric flask, and the volume was made up with methanol to get the concentration of 300 µg/mL of rasagiline and 12 µg/mL of riluzole. The intraday precision study was carried out by preparing drug solutions of the same concentration and analyzing it at three different times in a day **Table 10** and **11**.

TABLE 10: INTRADAY PRECISION DATA FOR RASAGILINE

Replicates	Absorbance	Simultaneous method		AUC	
		Absorbance	Conc. (µg/mL)	Absorbance	Conc. (µg/mL)
1	1	0.535	149.9	0.78	150
2	2	0.535	149.9	0.789	151.9
3	3	0.536	150.22	0.789	151.9
	Mean	0.535333	150.0067	0.786	151.2667
	Standard deviation	0.000577	0.184752	0.005196	1.096966
	% RSD	0.107849	0.123463	0.661088	0.725187

TABLE 11: INTRADAY PRECISION DATA FOR RILUZOLE

Replicates	Absorbance	Simultaneous method		AUC	
		Absorbance	Conc. (µg/mL)	Absorbance	Conc. (µg/mL)
1	1	0.552	12.0	0.96	12.0
2	2	0.553	12.3	0.965	12.7
3	3	0.554	12.6	0.965	12.7
	Mean		12.3		12.4
	Standard deviation	0.000567	0.15346	0.002786	1.0567
	% RSD	0.2364	0.3485	0.64889	0.3556

To determine interday precision the same procedure was followed for three different days by following the same procedure **Table 12** and **13**. The results were reported as % RSD.

TABLE 12: INTERDAY PRECISION DATA FOR RASAGILINE

Replicates	Absorbance	Simultaneous method		AUC	
		Absorbance	Conc. (µg/mL)	Absorbance	Conc. (µg/mL)
1	Day 1	0.535	149.9	0.78	150
2	Day 2	0.535	149.9	0.789	151.9
3	Day 3	0.536	150.22	0.789	151.9
	Mean	0.535333	150.0067	0.786	151.2667
	Standard deviation	0.000577	0.184752	0.005196	1.096966
	% RSD	0.107849	0.123163	0.661088	0.725187

TABLE 13: INTERDAY PRECISION DATA FOR RILUZOLE

Replicates	Absorbance	Simultaneous method		AUC	
		Absorbance	Conc. (µg/mL)	Absorbance	Conc. (µg/mL)
1	Day 1	0.552	12.0	0.96	12.0
2	Day 2	0.556	12.3	0.965	12.7
3	Day 3	0.554	12.6	0.965	12.7
	Mean		12.3		12.4
	Standard deviation	0.0005361	0.1348	0.002785	1.0647
	% RSD	0.3547	0.624	0.964	0.5826

Accuracy: The accuracy of an analytical procedure expresses the closeness of agreement between the value that is accepted either as a true conventional value or as an accepted reference value found. The recovery studies were carried out by adding different amounts (50%, 100%, and 150%) of the pure drug to the pre-analyzed formulation **Table 14** and **15**.

TABLE 14: ACCURACY DATA FOR RASAGILINE

Accuracy level	Amount added (µg/mL)	% recovery	Mean % recovery	% RSD
I (50%)	100.0	101.06±0.66	100.43%	0.523
II (100%)	200.0	100.10±0.56		
III (150%)	300.0	100.22±0.64		

TABLE 15: ACCURACY DATA FOR RILUZOLE

Accuracy level	Amount added (µg/mL)	% recovery	Mean % recovery	% RSD
I (50%)	100.0	99.57±0.64	100.01%	0.520
II (100%)	200.0	100.22±0.55		
III (150%)	300.0	100.25±0.32		

Ruggedness: Ruggedness expresses the variation within laboratories in analyzing the drugs in different days, by the different analyst, using different equipments, *etc.* The intermediate precision was performed for rasagiline and riluzole by a different analyst on a different day.

Preparation of Standard Solutions of Mixtures: The working standard solutions of rasagiline and riluzole were prepared as per the procedure mentioned in the precision method.

Determination: The resulting mixtures of samples are subjected to UV analysis by different analysts, and the obtained absorbance was recorded, and the % RSD of replicates was calculated. The results obtained were presented in **Tables 16** and **17**.

TABLE 16: RUGGEDNESS DATA FOR RASAGILINE AND RILUZOLE BY ANALYST 1

Concentration (µg/mL)		Absorbance/area			
		Simultaneous method		AUC	
Rasagiline	Riluzole	Rasagiline	Riluzole	Rasagiline	Riluzole
150	12	0.534	0.556	0.780	0.963
150	12	0.533	0.558	0.785	0.965
150	12	0.538	0.557	0.783	0.964
150	12	0.535	0.556	0.786	0.968
150	12	0.531	0.554	0.781	0.964
Mean		0.5342	0.5562	0.783	0.9648
Standard deviation		0.002588	0.001483	0.00255	0.001924
% RSD		0.484544	0.266674	0.325608	0.199372

TABLE 17: RUGGEDNESS DATA FOR RASAGILINE AND RILUZOLE BY ANALYST 2

Concentration (µg/mL)		Absorbance/area			
		Simultaneous method		AUC	
Rasagiline	Riluzole	Rasagiline	Riluzole	Rasagiline	Riluzole
150	12	0.531	0.555	0.781	0.962
150	12	0.538	0.553	0.783	0.963
150	12	0.536	0.556	0.783	0.965
150	12	0.534	0.551	0.786	0.968
150	12	0.539	0.552	0.782	0.963
Mean		0.5356	0.5534	0.783	0.9642
Standard deviation		0.003209	0.002074	0.001871	0.002387
% RSD		0.599209	0.37471	0.238931	0.247611

Limit of Detection and Limit of Quantification: Limit of detection (LOD) and limit of quantification (LOQ) were determined based on the standard deviation of response and the slope and were calculated by using the equation

$$LOD = 3 \times s/S \text{ and } LOQ = 10 \times s/S$$

Where s is the standard deviation of intercept and S is the slope of the line

RESULTS: Rasagiline and Riluzole were individually analyzed by UV-Visible spectrophotometric method using the solvent methanol. Optical characteristics such as λ_{max}, $E^{1\%}_{1cm}$, slope intercept, correlation coefficient, linearity and range, LOD and LOQ were observed as in **Table 18** and **19**.

TABLE 18: RASAGILINE CALIBRATION DATA

Parameters	Simultaneous method	AUC
λ_{max} (nm)	265.20	260.20-270.20
$E^{1\%}$ 1cm	31.026	30.652
Slope	0.0026	0.003
Intercept	0.0257	0.0052
Correlation coefficient	0.9995	0.9994
Linearity	50-350	50-350
LOD (µg/mL)	0.79	1.4
LOQ (µg/mL)	1.17	4.3

The mixture of rasagiline and riluzole was analyzed by UV-Visible spectroscopic method using simultaneous equation method and area under the curve method (AUC). These methods were validated according to the ICH guidelines. The observed results of the developed and validated methods in the present study suggest that these methods can be adopted for routine analysis of drugs simultaneously.

TABLE 19: RILUZOLE CALIBRATION DATA

Parameters	Simultaneous method	AUC
λ_{max} (nm)	263.40	258.40-268.40
$E^{1\%}$ 1cm	468.58	252.25
Slope	0.0546	0.0256
Intercept	0.0048	0.0024
Correlation coefficient	0.9995	0.9998
Linearity	4-20	4-20
LOD (µg/mL)	0.55	0.58
LOQ (µg/mL)	1.695	0.98

CONCLUSION: A physical mixture was prepared in the laboratories of Govt. College of Pharmacy as no combined dosage forms of rasagiline and riluzole are available in the market. As spectroscopic methods are highly powerful and convenient methods of analysis, in the present study, two different such methods were developed and validated for routine analysis of rasagiline and riluzole in their bulk and physical mixtures. In the simultaneous equation method, different wavelengths are selected to calculate their concentrations in both bulk and in combination.

The simultaneous equation method is extended to calculate the concentration as the area under the curve method at a wavelength of ±5 nm λ_{max} of rasagiline and riluzole. These developed methods are economical, accurate, and precise. From the observations of both methods developed and validated, they may be used for routine analysis of rasagiline and riluzole simultaneously at the industrial level in their dosage forms.

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